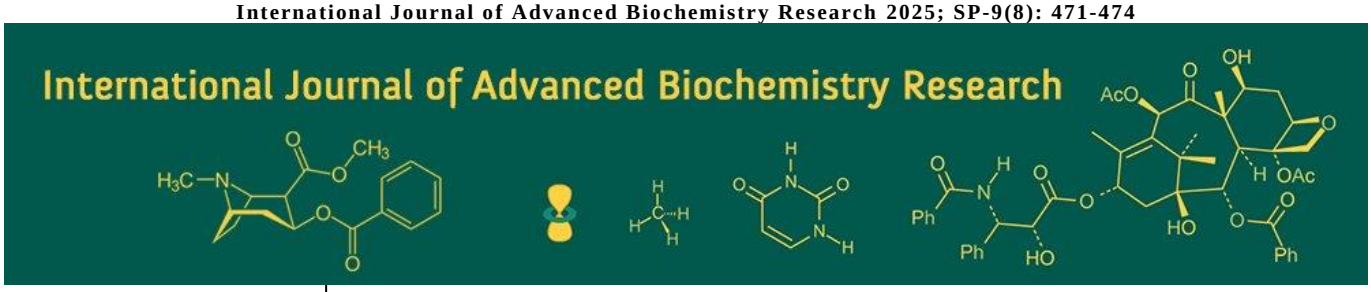

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Identification of sources of resistance to post-flowering stalk rot in short duration maize (*Zea mays* L.) inbred lines with temperate background

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Abstract
Post-Flowering Stalk Rot (PFSR) is a major disease limiting maize (*Zea mays L.*) production, especially under moisture stress and high planting density. The disease, predominantly caused by *Macrophomina phaseolina* and *Fusarium verticillioides*, leads to stalk lodging, premature senescence, and significant yield losses. Developing resistant cultivars is a critical approach to managing this disease.

In the present study, 37 short-duration maize inbred lines with temperate background were evaluated for resistance to PFSR under artificial epiphytotic conditions using the standardized toothpick inoculation method. Disease severity was assessed at physiological maturity using a 1-9 rating scale.

Out of 37 genotypes, 7 lines including KDMB-134, KDMB-143, KDMB-212, KDMB-412, KDMB-93-1, KDMB-225, and KDMB-20264 were classified as resistant lines (score ≤ 3.0). Twenty two lines showed moderate resistance (score 3.1-5.0), while 8 lines were moderately to highly susceptible. Significant variability in disease response was observed, indicating a strong genetic basis for resistance. The identified resistant lines can serve as valuable donors in breeding programs aimed at developing stalk rot-tolerant hybrids. These findings provide a foundation for future genetic dissection, QTL mapping, and resistance breeding against PFSR in maize.

Keywords: Maize, Charcoal rot, *Macrophomina phaseolina*, disease resistance, inbred lines, toothpick inoculation, PFSR

Introduction
Maize is one of the most significant cereal crops in the world which is widely grown for food, feed, and industry. With an average yield of about 3.5 tonnes per hectare, maize is farmed on more than 12 million hectares in India (Indiastat, 2024) [6]. Numerous biotic and abiotic stressors continue to limit maize output despite rising demand and growing cultivation. Post-Flowering Stalk Rot (PFSR) is one of the biotic stressors that has become a significant limiting factor in a variety of agroclimatic zones. PFSR complex often afflict maize in the post-flowering to pre-harvest phases. *Cephalosporium acremonium* (Black Bundledisease), *Macrophomina phaseolina* (charcoal rot), *Fusarium verticillioides* (Fusarium stalk rot), and *Cephalosporium maydis* (late wilt) are the main causative pathogens. These infections cause tissue discolouration and internal stalk rot, which eventually interfere with the movement of nutrients and water, leading to early mortality, lodging, and significant output losses. (Khokhar *et al.*, 2014) [8].

Because of its vigorous colonisation in dry and moisture-stressed conditions, *Macrophomina phaseolina*, the cause of charcoal rot, is thought to be the most destructive of them. According to recent estimates, yield losses from this disease can vary from 10% to 42% (2021), with increased severity it reaches as high as 22.3% to 63.5% occasionally resulting in complete crop failure.

The pathogen's ability to persist in soil for up to three years after host plant breakdown adds to its threat to maize productivity. Elevated temperatures and decreased soil moisture levels make maize plants more susceptible to *M. Phaseolina* infection. (Laxmi Sravya *et al.*, 2023) [10]. Despite its widespread and harmful effects, little is known about genetic resistance to PFSR, and no efforts have been made to develop resistant cultivars. (Banoth *et al.*, 2021) [1]. Breeding for genetic resistance is still the most economical, environmentally responsible, and

sustainable approach, (Harish *et al.*, 2023) [5] despite the fact that biological control methods have been the subject of numerous studies (Chandra Nayaka *et al.*, 2010; Wu *et al.*, 2015; Lu *et al.*, 2020; Jambhulkar *et al.*, 2022) [3, 13, 9, 7].

The creation of robust, disease-resistant hybrids is severely hampered by this dearth of knowledge. Effective breeding tactics require a thorough grasp of the PFSR resistance inheritance pattern. But thus far, this feature has received little attention (Bhaskar *et al.*, 2023) [2]. Under simulated epiphytotic conditions, different genotypes of maize have been evaluated against the causative diseases to aid in the development of PFSR resistant hybrids (Hooda *et al.*, 2012) [4].

Genotypes of maize differ greatly in their genetic resistance to stalk rots. Although many resistance sources have been documented, nothing is known about the application of short-duration, PFSR-resistant inbred lines, particularly those with a temperate origin. Finding such resistant genotypes could be very beneficial for breeding, since short-duration lines have an agronomic benefit in regions with terminal drought stress or double-cropping systems.

In order to assess the resistance levels of 37 short-duration maize inbred lines of temperate provenance, the current study was conducted using artificial inoculation in a field setting. Finding potential genotypes with PFSR resistance that might be used as donor parents in resistance breeding initiatives to increase maize's resistance to stalk rot was the goal.

Materials and Methods

The field experiment was conducted during *rabi* 2024-25 at the Maize Research Centre, Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, located at 17°19′N latitude, 79°23′E longitude, and 542.6 meters above mean sea level. The experiment was laid out in a Randomized Complete Block Design (RCBD) with two replications. Each genotype was sown in two rows, following a spacing of 60 cm between rows and 20 cm between plants.

The study comprised 37 short-duration maize inbred lines with temperate background, along with two checks (CM-600 and JCY-2-7). The complete list of genotypes is provided in Table 1. These inbreds were evaluated under artificial disease pressure for their reaction to Post-Flowering Stalk Rot (PFSR).

The test pathogen was *Macrophomina phaseolina*, which causes charcoal rot. Potato dextrose agar (PDA) medium was used to isolate and cultivate the pathogen. To guarantee consistent fungal colonisation, sterile wooden toothpicks were autoclaved after being soaked in sterile water and then incubated on PDA culture plates with *M. Phaseolina* for five to seven days. Using the standardised toothpick approach, inoculation was carried out during the silking stage, which occurred 50-55 days after sowing. Using a sterile jabber, a little vertical incision, 2-3 cm deep, was made in the stalk's basal internode. In order to promote infection, the colonised toothpicks were placed into the wounds such that the interior tissues were in direct touch with the fungal mycelium.

After the plants reached physiological maturity, disease symptoms such lodging, pith necrosis, and discoloured stalks were noted. Splitting the stalk longitudinally and visually rating internal damage on a scale of 1 to 9—lower scores denoting resistance and greater scores denoting susceptibility—was how the disease severity was assessed. (Sharma and Payak, 1983) [12] The scoring scale and corresponding disease reactions are summarized in Table 2.

Table 1: Short-duration maize inbred lines that were screened to identify genotypes with resistance or susceptibility to post-flowering stalk rot (PFSR).

S. No	Genotype	S. No	Genotype
1.	KDMB-187	9.	KDMB-151
2.	KDMB-1018	10.	KDMB-200-1
3.	KDMB-568	11.	KDMB-338
4.	KDMB-941-1	12.	KDMB-134
5.	KDMB-139	13.	KDMB-72
6.	KDMB-22460	14.	KDMB-1070-1
7.	KDMB-542	15.	KDMB-474
8.	KDMB-351	16.	KDMB-46-3-1
17.	KDMB-15	29.	KDMB-93-1
18.	KDMB-12	30.	KDMB-5-2
19.	KDMB-488	31.	KDMB-143-1
20.	KDMB-184270	32.	KDMB-2068
21.	KDMB-376-2	33.	KDMB-145
22.	KDMB-1	34.	KDMB-225
23.	KDMB-143	35.	KDMB-2046
24.	KDMB-375-1	36.	KDMB-10147
25.	KDMB-212	37.	KDMB-20264
26.	KDMB-2		Checks
27.	KDMB-412	1.	CM 600
28.	KDMB-407	2.	JCY-2-7

Table 2: Disease rating scale

Disease rating scale	Disease severity percentage (%)	Disease reaction
1	Healthy or trace/slight discoloration at the site of inoculation	Immune reaction
2	Up to 50% of the inoculated internode is discoloured	Resistant (Score: ≤ 3.0)
3	51-75% of the inoculated internode is discoloured	
4	76-100% of the inoculated resistant internode is discoloured	Moderately resistant (Score: 3.1-5.0)
5	Less than 50% discoloration of the adjacent internode.	
6	More than 50% discoloration of the adjacent internode	Moderately susceptible (Score: 5.1-7.0)
7	Discoloration of three internodes.	
8	Discoloration of four internodes.	Susceptible (Score: ≥7.0)
9	Discoloration of five or more internodes and premature death of the plant.	

Results and Discussion

To assess their resistance to Post-Flowering Stalk Rot (PFSR), 37 short duration maize inbred lines with a temperate background were screened in artificial epiphytotic settings. At the silking stage, the standardised toothpick inoculation method was used for screening, and a scale of 1 to 9 was used to determine the disease severity at physiological maturity (Table 3). A significant amount of diversity in disease response was seen from the genotypes'

diverse responses to PFSR. The lines were categorised into four groups based on visual scoring:

- Resistant (score 1-3): 7 genotypes
- Moderately Resistant (score 3.1-5): 22 genotypes
- Moderately Susceptible (score 5.1-7): 4 genotypes
- Susceptible (score 7.1-9): 4 genotypes

The findings revealed that certain inbred lines showed a clear phenotypic expression of resistance to PFSR under field conditions. The genotypes KDMB-134, KDMB-143, KDMB-212, KDMB-412, KDMB-93-1, KDMB-225, and KDMB-20264 exhibited resistance with a mean disease reaction score ≤ 3. These lines showed minimal internal discolouration and lesion development. Lines such as KDMB-1018, KDMB-139, KDMB-22460, KDMB-351, KDMB-151, KDMB-1070-1, KDMB-200-1, KDMB-72, KDMB-474, KDMB-46-3-1, KDMB-15, KDMB-488, KDMB-187, KDMB-184270, KDMB-376-2, KDMB-375-1, KDMB-2, KDMB-407, KDMB-5-2, KDMB-143-1, KDMB-2046 and KDMB-2068 were classified as moderately resistant with disease scores ranging between 3.1 and 5. In contrast to genotypes, KDMB-568, KDMB-941-1, KDMB-542, KDMB-338, KDMB-12, KDMB-1, KDMB-145, and KDMB-10147 displayed higher lesion lengths and discolouration and were rated as moderately susceptible to susceptible (scores between 5.1 and 8).

Some inbred lines may have innate genetic resistance, as indicated by the observed difference in disease response. The study's artificial inoculation technique was successful in generating consistent and homogeneous disease pressure, reducing environmental influence and facilitating precise genotype discrimination. In studies on maize pathology, this approach has received extensive validation (Payak and Sharma, 1985) [11]. In vulnerable genotypes, environmental conditions like high temperatures and post-flowering moisture stress may have made the disease even worse.

There is substantial practical significance in identifying resistant genotypes. In resistant breeding initiatives aimed at producing short-duration maize hybrids appropriate for areas vulnerable to terminal drought and stalk rots, these lines can be useful donor parents. Incorporating them into breeding pipelines may increase the genetic foundation for disease resistance while simultaneously enhancing stalk integrity and yield stability. In order to support marker-assisted selection for PFSR resistance, these resistant lines may also be advanced for molecular characterisation and QTL mapping.

Table 3: Average disease reaction of different maize inbreds

S. No	Genotype	Disease mean score on (1-9, scale)	Disease reaction
1	KDMB-187	3.3	MR
2	KDMB-1018	4.1	MR
3	KDMB-568	5.7	MS
4	KDMB-941-1	7.6	S
5	KDMB-139	3.5	MR
6	KDMB-22460	4.3	MR
7	KDMB-542	5.7	MS
8	KDMB-351	3.3	MR
9	KDMB-151	5	MR
10	KDMB-200-1	3.3	MR
11	KDMB-338	5.2	MS
12	KDMB-134	1.6	R
13	KDMB-72	4.3	MR
14	KDMB-1070-1	3.2	MR
15	KDMB-474	4.2	MR
16	KDMB-46-3-1	3.8	MR
17	KDMB-15	4.4	MR
18	KDMB-12	6	MS
19	KDMB-488	4.2	MR
20	KDMB-184270	4.3	MR
21	KDMB-376-2	4.6	MR
22	KDMB-1	7.7	S
23	KDMB-143	2.9	R
24	KDMB-375-1	4.1	MR
25	KDMB-212	2.5	R
26	KDMB-2	4.5	MR
27	KDMB-412	2.4	R
28	KDMB-407	4.9	MR
29	KDMB-93-1	2.3	R
30	KDMB-5-2	3.3	MR
31	KDMB-143-1	4.6	MR
32	KDMB-2068	3.5	MR
33	KDMB-145	7.6	S
34	KDMB-225	2.5	R
35	KDMB-2046	4.2	MR
36	KDMB-10147	7.5	S
37	KDMB-20264	1.9	R
	Checks		
	JCY-2-7	2.2	R
	CM 600	8.5	S

Conclusion

In this study, 37 short duration maize inbred lines with a temperate origin were found to have varying levels of resistance to Post-Flowering Stalk Rot (PFSR). The classification of genotypes into resistant and susceptible groups was made possible by the observation of a clear spectrum of disease reactions following controlled artificial inoculation. There was significant genetic heterogeneity in the examined material, as seen by the nine genotypes that were found to be resistant and the several others that displayed moderate resistance.

In maize breeding projects aimed at creating hybrids with enhanced resistance to stalk rot, these resistant genotypes can be promising sources of resistance. The results also lay the groundwork for further research into resistance breeding, molecular characterisation, and genetic mapping. By integrating these lines into conventional breeding pipelines, yield losses from PFSR will be reduced and resilient maize cultivars appropriate for stress-prone areas will be developed.

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Competing Interests

"Authors have declared that no competing interests exist.".

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